

Oseltamivir-resistant influenza?

Maki Kiso and colleagues' Article in this week's *Lancet* is a timely wake-up call. Highly pathogenic avian influenza viruses (H5N1 strain) have just become endemic in Asian domestic fowl, spread by wild birds, and probably cannot be eradicated.¹ During 2004, these viruses were transmitted from infected chickens or ducks directly to human beings causing severe and sometimes fatal disease, and thus have the potential to ignite a worldwide pandemic. Widespread influenza is the deadliest plague in human history, and transmission of avian influenza to human beings probably started the global pandemic of 1918, which killed 50 million people.

Several quite likely scenarios can lead to the 2004 avian virus becoming more transmissible from human being to human being. If the virulent avian influenza recombines in people with a human influenza, or recombines in the pigs that already harbour these human viruses, a terrifyingly lethal strain will present an immediate pandemic threat to human beings. The recent announcement by Chen Hualan, chief of the China National Avian Influenza Reference Laboratory, that avian influenza has been isolated from pigs, although awaiting confirmation, adds to the concern of recombination between human and avian strains in pigs.²

Preparedness needs a multi-pronged approach to avert the pandemic, control outbreaks, and ultimately prevent high mortality during international spread. Although vaccination would be the first line of defence, no vaccine against the H5N1 influenza is yet available. WHO concluded that any vaccine supplies would be inadequate during a pandemic. Thus we are fortunate that new approaches to drug development, based on understanding the structure and function of viral neuraminidase and its interaction with host receptor,³ have led to several effective and non-toxic anti-influenza drugs: the neuraminidase inhibitors.

These drugs block the receptor-cleaving enzyme of influenza⁴ that the virus uses to release itself from an infected cell to spread further. Structural studies of the influenza neuraminidase protein led to the design of a molecule, zanamivir, which mimics the virus' natural substrate, interacting with the active site of neuraminidase and blocking its action. Without neuraminidase, the infection cannot progress. Zanamivir is given by oral inhalation, delivering drug directly to the respiratory tract. The drug proved to be clinically effective, with virtually no side-effects.⁵ Right on the heels of zanamivir, oseltamivir was developed—promising news since the drug is effective orally and simple to use. Both zanamivir and oseltamivir reduce the duration and severity of illness, reduce viral shedding, and are effective prophylactics.⁵⁻¹⁰

Neuraminidase inhibitors are effective against all neuraminidase subtypes, and, therefore, against all strains of influenza; this effect is a key point in pandemic preparedness and an important advantage over the adamantanes or M2 inhibitors (amantadine and rimantadine), which are only effective against influenza A. Neuraminidase inhibitors are effective against the neuraminidase from the virus that caused the 1918 pandemic¹¹ and the avian viruses that caused outbreaks

in 1997–99.^{12,13} The 2004 avian influenza H5N1 strains, while resistant to the M2 inhibitors,¹⁴ are sensitive to neuraminidase inhibitors.¹⁵

In the event of a new influenza strain in an unvaccinated community, we can now envision stockpiling neuraminidase inhibitors, relying on these drugs to reduce mortality. Optimism can be derived from the fact that until now there has been very little reason for concern about the development of resistance to neuraminidase inhibitors. No de-novo resistance to neuraminidase inhibitors has been found to date,¹⁶ although primary resistance to M2 inhibitors is not rare.¹⁷ For M2 inhibitors, resistant virus is generated easily in up to 30% of patients, and these resistant viruses are virulent and transmissible.¹⁸

The structure-based design of zanamivir seemed to have saved the neuraminidase inhibitors from this fatal flaw. In fact, no resistant virus has been isolated from immunocompetent people given zanamivir. For oseltamivir, the published frequency of post-treatment viruses that have neuraminidase resistance is higher. Whereas only about 0.4% of treated adults harboured viruses with resistant neuraminidases, when children were treated this number jumped to at least 4%.¹⁹

Kiso and colleagues' study challenges our complacency about neuraminidase-inhibitor resistance. Of 50 children in Japan treated with oseltamivir, nine (18%) harboured viruses with mutations in the neuraminidase gene that conferred drug resistance. Where are these mutations in the neuraminidase proteins? They are exactly where predicted by in-vitro studies

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in which the possibility of emergence of resistance to the different neuraminidase inhibitors was compared.^{20,21}

Six children in Japan had viruses with mutations of arginine to lysine at aminoacid 292 of the neuraminidase molecule. This arginine forms part of the active site of the enzyme, where the neuraminidase inhibitors also bind, blocking the enzyme's activity. The change to lysine prevents the movement of other aminoacids in the active site that create a pocket into which the bulky side-chain of oseltamivir fits. If the pocket cannot be formed, oseltamivir cannot fit, leading to resistance.²² The new mutation at position 294, described by Kiso and colleagues, might also affect movement of these aminoacids. Viruses from two other children had mutations at glutamic acid 119. This mutation, in a residue that forms part of the neuraminidase active-site framework, has previously been recorded in oseltamivir-treated patients. If the high percentage of mutants in Kiso's study is a general occurrence, then mutations could occur in children far more frequently than has been observed.¹⁹

Are the oseltamivir-resistant viruses transmissible and pathogenic? If we are very lucky, they may have a growth disadvantage, or, for other reasons, be less virulent or transmissible. This is an important question left open by Kiso that needs to be addressed. If resistant variants are transmissible and pathogenic, then the widespread use of oseltamivir in a pandemic situation raises concerns. Generally, neuraminidase mutations have led to viruses with reduced pathogenicity in animal models^{22,23} because the mutations cause defects in an important enzyme. There is no documented transmission of an oseltamivir-resistant virus in human beings, but the frequent emergence of resistance mutations in Kiso's study suggests that this is only a matter of time.

What can be done? In view of the threat of a pandemic, we need to heighten awareness of the critical importance of neuraminidase inhibitors for our arsenal. Stockpiles of these drugs are a key piece of preparedness and would be critical to an effective response. For these reasons, it is vital to understand more about which features of neuraminidase inhibitors might discourage the emergence of resistance. Whereas zanamivir seems preferable in terms of development of resistance, the route of delivery could be problematic for some populations. We need more information on the emergence of resistance, especially in oseltamivir-treated patients, and we urgently need to know whether resistant variants, such as those identified in Kiso's study, are transmissible. The development of the neuraminidase inhibitors has been a true success story: protein structural analysis directly applied to preventing and treating a major infectious threat. Let us take Kiso and colleagues' study as an energising mandate to learn more about the incidence and mechanisms of resistance to the neuraminidase inhibitors, so that appropriate strategies can be developed for their use during the next pandemic.

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I declare that I have no conflict of interest.

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